AD_____

Award Number: W81XWH-04-1-0172

TITLE: ATM Heterozygosity and the Development of Radiation-Induced Erectile Dysfunction and Urinary Morbidity Following Radiotherapy for Prostate Cancer

PRINCIPAL INVESTIGATOR: Jamie A. Cesaretti, M.D.

CONTRACTING ORGANIZATION: Mount Sinai School of Medicine
New York, New York, 10029

REPORT DATE: February 2008

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
01-02-2008	Annual	1 FEB 2007 - 31 JAN 2008

4. TITLE AND SUBTITLE

ATM Heterozygosity and the Development of Radiation-Induced Erectile Dysfunction and Urinary Morbidity Following Radiotherapy for Prostate Cancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0172

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jamie A. Cesaretti, M.D.

E-Mail: jamie.cesaretti@frogdocs.com

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Mount Sinai School of Medicine
New York, New York, 10029

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of this training grant project is to determine whether the prevalence of ATM carriers among prostate cancer patients treated with radiotherapy that develop erectile dysfunction and urinary morbidity is greater than the prevalence of ATM heterozygosity among patients that do not develop this complication. Regardless of the scientific outcome of the proposal the PI will be left with a vast experience in translational research from which to form new hypotheses and research strategies as he begins his career as an independent physician scientist. To assure a well-rounded experience, the school of medicine will insure that the PI will participate for the first two years of the funded period in Mount Sinai's rigorous clinical research training program. The NIH sponsored program will give the PI formal instruction in Clinical Research and Policy Evaluation, Epidemiology and Biostatistics, Basic Science for the Clinical Investigator, Cultural, Illness, and Community Health Outcomes, Behavioral Medicine, and Ethical Issues in Clinical Research. Also the PI, while at Mount Sinai, will make significant progress in establishing collaborative relationships with well-established prostate cancer researchers and will continue this approach in order to expand the scope of the outlined proposal throughout the funding period of this grant.

15. SUBJECT TERMS
Radiotherapy, Mutation Screening, Functional Analysis

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRMC
U	U	U	UU	27	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

INTRODUCTION:

Many patients with prostate cancer treated with radiotherapy develop erectile dysfunction and urinary morbidity induced by exposure to a high dose of radiation. In some cases there are explanations for these reactions, such as doses to large volumes of normal tissue or pre-existing medical conditions such as diabetes or collagen vascular diseases. However, there exists an important subset of patients with no clear explanation for excessive post-treatment morbidity and the potential for a genetic basis must be considered. The purpose of this study is to investigate whether the ATM gene plays a role in this radiation sensitivity. This gene was selected, as the protein it encodes, plays a critical role in the response of cells to irradiation and the repair of radiation-induced damage. Furthermore, cells possessing one mutated copy of this gene are radiosensitive. In addition, the results of a pilot study screening breast cancer patients are supportive of the hypothesis that patients who are carriers of an ATM mutation are more likely to develop radiation-induced complications.

The principal goal of this project is to determine whether men who inherit a mutated copy of the ATM gene are more prone to the development of radiation-induced erectile dysfunction and urinary morbidity. This has largely been accomplished through comprehensive screening of the ATM gene for germline mutations. Several correlations have been found between radiosensitivity and ATM heterozygosity, this indicates that possession of a mutated copy of the ATM gene results in susceptibility to complications for prostate cancer radiotherapy patients (See table 1 and Figure 1).

Figure 1. Int. J. Radiation Oncology Biol. Phys., Vol. 61, No. 1, pp. 196–202, 2005

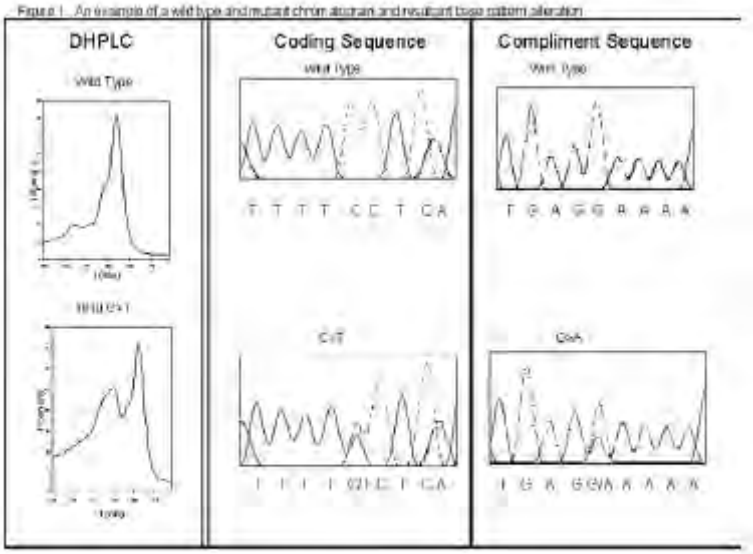


Fig. 1. An example of a wild-type and mutant chromatogram and resultant base pattern alteration.

Table 1. Int. J. Radiation Oncology Biol. Phys., Vol. 61, No. 1, pp. 196–202, 2005

Table 4. Each patient with toxicity, genetic, comorbid, and follow-up data

Patient (#)	*ATM* alteration	Prospective erectile decline	Last follow-up IIEF-5	Rectal bleeding	Urinary quality of life	D_{90} ‡ (Gy)	Comorbidities	Follow-up (months)
1	4473C>T, 149.1F>F	No	24	No	1	184	CAD	21
2		No	18	No	4	192		36
3	4578C>T, 1526P>P; 5557G>A, 1853D>N	Yes	2	RTOG 1	6	180		67
4		No	20	No	3	208	Tob	37
5		No	16	No	2	205	Tob	29
6		No	24	No	1	165		36
7		*	10	No	0	191		70
8		No	†	No	2	220		49
9	1810C>T, 604P>S	Yes	16	No	6	208		19
10	378T>A, 126D>E; IVS7-8insT; 1176C>G, 392G>G	Yes	1	No	2	197	DM	12
11	2685A>G, 895L>L; 2614C>T, 872P>S	Yes	1	RTOG 1	1	205		40
12	IVS38-8T > C	No	24	No	1	159		60
13		*	23	No	2	174	DM, CAD	31
14		No	1	No	3	210	CAD	20
15	IVS38-8T>C	No	19	No	4	164	Tob	39
16		No	14	No	0	183		59
17		*	5	No	0	169		44
18		No	22	No	2	220		40
19		No	12	No	2	206		26
20		*	21	No	2	199	Tob	37
21		*	2	No	2	174	DM, CAD	25
22	198A>C, 66K>K	*	1	No	1	217		40
23		No	23	No	1	160		25
24		Yes	9	No	2	184		39
25		*	6	No	4	218		32
26	4388T>G, 1463F>C; 1810C>T, 604P>S	*	2	RTOG 2	2	209	CAD	13
27		No	15	No	4	205		32
28	5071A>C, 1691S>R	Yes	1	RTOG 2	2	192		45
29	3161C>G, 1054P>R	No	19	No	2	197		27
30	IVS62+8A>C	No	19	RTOG 1	0	217	CAD	47
31	4578C>T, 1526P>P	Yes	8	No	0	193		26
32	2038T>C, 680F>L	No	19	RTOG 1	0	219		31
33		No	24	No	2	162		71
34		*	3	No	0	168	CAD	69
35	5557G>A, 1853D>N	No	20	No	0	186		58
36		No	18	No	1	197		43
37	IVS22-6T>G	No	22	No	3	210		29

Abbreviations: CAD = coronary artery disease; DM = diabetes mellitus; RTOG = Radiation Therapy Oncology Group; Tob = active smoker.

* Patient had a suboptimal erectile function before implant.

† Patient did not fill out IIEF-5.

‡ Dose to 90% of the prostate gland via brachytherapy.

In addition, a determination was made as to the pathogenic consequences of each ATM mutation through the use of functional studies that examined the ability of the ATM protein to act normally in cells from patients who are carriers of a mutation in this gene. These results were largely negative and remain unpublished because of lack of clinical application and interest. I have included a table 2 for direct reference and an extended appendix F with the results in their entirety.

Table 2.

Table 1. Functional Assays of Lymphoblastoid Cells Derived from Subjects Possessing *ATM* Variants

Cell Line	Radio-sensi-tive Yes/No	Nucleotide Change	Amino Acid Substitu-tion	ATM level	Phospho-p53 0.5 hr	Phospho-p53 2 hr	Normalized α-value
MS01-33	no	4138 C>T	1380 H>Y	1.1+0.6	5.1+4.4	5.4+3.0	1.2±0.2
MS01-30	No	IVS5-7 C>T 378 T>A 4578 C>T	N/A 126 D>E 1526 P>P	0.5+0.3	2.0+1.7	4.5+4.0	1.0±0.4
MS01-39	Yes	5557 G>A 5558 A>T	1853 D>N 1853 D>V	1.3±0.9	1.4±1.0	2.7±0.4	1.1±0.5
MS01-45	No	5557 G>A	1853 D>N	0.4±.04	1.4±.0.6	1.6±1.0	1.3±0.1
MS01-51	Yes	IVS5-7C>T 378 T>A	N/A 126 D>E	0.7±0.5	2.5±2.6	9.5±4.5	0.5±.2
MS01-37	Yes	378 T>A 1176 C>G 4138 C>T	126 D>E 392 G>G 1380 H>Y	1.6±0.2	2.1±1.2	2.0±1.2	1.4±0.4
MS01-67	Yes	4578 C>T	1526 P>P	0.5±.09	4.6±0.8	10.7±3.7	1.2±0.1
MS01-65	No	5557 G>A	1853 D>N	1.1±0.5	2.7±1.2	10.1+4.0	1.2±0.3
Ms01-53	No	378 T>A 1176 C>G	126 D>E 392 G>G	1.0±.0.1	2.5±0.8	6.5±2.1	0.8±0.3
MS01-07	No	4917 G>A 5557 G>A 5558 A>T	1639 P>P 1853 D>N 1853 D>V	0.8±0.5	0.9±0.7	2.0±1.7	0.5±0.3
MS01-37	Yes	378 T>A 1176 C>G 4138 C>T	126 D>E 392 G>G 1380 H>Y	1.6+0.2	2.1+1.2	2.0+1.2	1.4±0.2
MS02-13	YES	378 T>A 6176 C>T	126 T>A 2059 T>I	1.0+0.2	2.0+1.3	5.1+3.7	1.2±0.2
MS02-73	YES	IVS62+8 A>C	N/A	0.8+0.3	4.5+4.0	3.8+1.3	0.8±0.3
MS01-87	YES	5071 A>C	1691 S>R	1.0+0.5	2.3+1.3	5.0+2.0	0.8±0.1
MS01-03	NO	2614 C>T	872 P>S	0.7+0.2	1.1+0.8	1.5+0.4	1.2±0.6

		2685 A>C	895 L>L				
MS01-35	NO	1229 T>C	410 V>A	0.9+0.6	2.9+0.1	5.7+3.7	1.1±0.2
MS02-34	YES	915 G>C	25 R>P	2.0+1.5	1.5+0.6	1.8+1.1	1.3±0.5
MS02-05	YES	NONE	N/A	0.7+0.4	3.1+3.7	4.6+3.7	1.1±0.3
MS03-13	YES	NONE	N/A	0.7+0.1	3.6+1.2	7.9+3.8	0.9±0.5
MS03-48	YES	NONE	N/A	0.5+0.3	2.4+1.4	6.8+2.1	1.3±0.1

Table 2. Functional Assays of *ATM* Homozygoyte and *ATM* Heterozygote Lymphoblastoid Cell Lines

Cell Line	Homozygote or Heterozygote	ATM Level	Phospho p53 0.5 hr	Phospho p53 2 hr	Normalized α-value for radiation survival curve
8388	heterozygote	0.7±0.6	1.6±0.2	6.7±2.3	1.5±0.2
8925	heterozygote	0.7±0.8	1.9±0.4	5.1±0.1	1.4±0.2
8928	heterozygote	0.8±0.3	3.8±3.5	3.5±2.7	1.7±0.2
9579	heterozygote	0.5±0.3	2.3±1.3	2.6±0.3	1.1±0.3
2781	heterozygote	0.7±0.5	3.2±0.6	4.5±4.1	1.6±0.2
9588	heterozygote	0.5±0.5	6.1±4.0	6.9±2.8	1.2±0.3
8436	homozygote	0.04±0.06	2.9±1.2	2.8±0.4	1.8±0.3
9581	homozygote	0.08±0.02	1.5±1.7	4.0±1.6	2.0±0.3
9582	homozygote	0.05±0.02	2.0±4.4	2.1±0.4	2.2±0.3
2782	homozygote	0.08±0.05	2.1±3.1	3.1±1.3	2.1±0.3
1525	homozygote	0.05±0.02	2.6±1.1	3.1±1.2	1.8±0.2
11254	homozygote	0.09±0.06	1.8±0.1	2.5±0.9	2.3±0.3
9586	homozygote	0.24±0.22	1.7±1.0	4.3±1.9	1.8±0.4
13328	homozygote	0.13±0.09	0.6±0.5	2.1±1.3	2.1±0.3

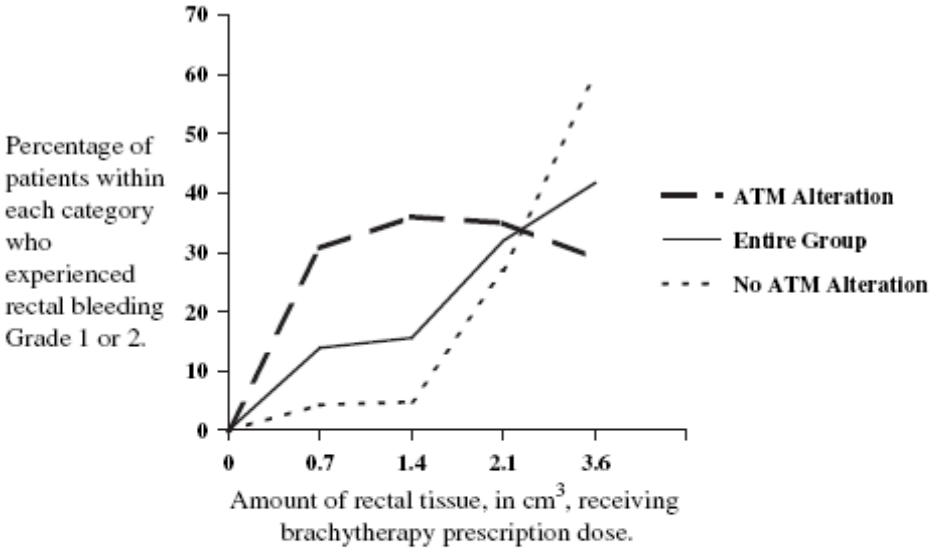
This project represents the first study to use the powerful DHPLC mutation screening technique to investigate the association between possession of a mutated ATM gene and both erectile dysfunction and the entire clinical course of a patient's urinary morbidity after treatment with radiation for prostate cancer. It is also the first study to examine whether there is a correlation between the presence of a mutation, development of a radiation-induced complication, and impairment of ATM protein function based upon cellular and molecular analyses. To this end or group expanded the initial hypothesis of clinical correlation between side effects and genetic findings to the incidence of rectal bleeding. Table 3 contains a summary of patient results and our findings.

Table 3. Cesaretti JA, Stock RG, Atencio DP, Peters SA, Peters CA, Burri RJ, Stone NN, Rosenstein BS. "A genetically determined dose-volume histogram predicts for rectal bleeding among patients treated with prostate brachytherapy." Int J Radiat Oncol Biol Phys. 2007 May

Table 4. *ATM* variants identified in 48 prostate cancer patients

Patient no.	Nucleotide location	Codon	Mutation type	Amino acid change
1–10	5557G→A	1853	M	D→N
11	167T→C	54	S	Y→Y
12	1810C→T	604	M	P→S
13	198A→C	66	S	K→K
14	2038T→C	680	M	F→L
15	2119T→C	707	M	S→L
16	2362A→C	788	M	S→R
17	2685A→G	895	S	L→L
	2614C→T	872	M	P→S
18	3161C→G	1054	S	P→R
19	378T→A	126	M	D→E
	415G→A	139	M	A→T
20	378T→A	126	M	D→E
	1176C→G	392	S	G→G
	IVS7-8insT	N/A	N/A	N/A
21	5557G→A	1853	M	D→N
	IVS38-8T→C	N/A	N/A	N/A
	IVS62+8A→C	N/A	N/A	N/A
22	1810C→T	604	M	P→S
	4388T→G	1463	M	F→C
23	4473C→T	1491	S	F→F
24–26	4578C→T	1526	S	P→P
27	5557G→A	1853	M	D→N
	4578C→T	1526	S	P→P
28	5557G→A	1853	M	D→N
	IVS38-8T→C	N/A	N/A	N/A
29	5557H	1853	M	D→N
	IVS38-8T→C	N/A	N/A	N/A
30	5558A→T	1853	M	D→V
31	5793T→C	1931	S	A→A
32	2572T→C	858	M	D→E
	9200C→G	N/A	N/A	N/A
33–45	IVS62+8A→C	N/A	N/A	N/A
46	735C→T	245	S	V→V
47, 48	IVS38-8T→C	N/A	N/A	N/A

Abbreviations: S = synonymous; M = missense; N/A = not available.



Number of	36	32	28	12
ATM Alteration	4 / 13	4 / 11	6 / 17	2 / 7
No ATM	1 / 23	1 / 21	3 / 11	3 / 5
Fisher's Exact	p=0.05	p=0.04	p=1	p=0.56

Fig. 2. Incidence of Grade 1 or 2 rectal bleeding (%) in the entire group of 108 patients given brachytherapy for prostate cancer and according to their *ATM* gene status.

Table 5. Univariate analysis of the distribution of variables between patients possessing a variant in *ATM* ($n = 48$) and the patients carrying two wild-type alleles ($n = 60$) that have been previously described to predict for both toxicity and PSA recurrence

Variable	ATM variant (+)	ATM variant (−)	*p*
Gleason sum	6 ± 0.4	6 ± 0.8	0.73*
PSA (ng/mL)	7.7 ± 5	7.2 ± 5.8	0.39*
Stage (T1 vs. T2) (*n*)	26 vs. 22	39 vs. 21	1†
BED	207 ± 26	200 ± 25	0.24*
Age (y)	64 ± 8	64 ± 8	0.71‡
Follow-up (mo)	43 ± 22	47.5 ± 22	0.35‡
Hormone therapy	35	45	1†
Addition of EBRT	21	22	1†
Smoker	31	37	1†
Diabetes	2	8	1†
Hypertension	31	33	1†
Coronary artery disease	15	12	1†
African American	15	17	1†

BODY:

My annual report covers the period from 2/1/07 to 1/31/08. I successfully completed the Mount Sinai Clinical Research Training Program, which is sponsored by an NIH K30 Clinical Research Curriculum Award, on 5/30/06. In addition to the training plan, regarding the Clinical Research Training Program, I completed additional coursework offered by Mount Sinai Medical School and was conferred a masters degree in Clinical Research on May 30, 2006.

I spent most of the year writing many scientific articles based upon the Mount Sinai experience using low dose rate brachytherapy for the treatment of prostate cancer in addition to other research efforts directed at the treatment of both lung and spine cancers. The articles are referenced in appendix A as reportable outcomes and parts of those articles directly of interest to the aims of the grant are referenced above. In addition to the publication of articles I have with my mentor received funding as a co-investigator for a study entitled," Genome-Wide Association Study to Identify SNPs and CNPs Associated with Development of Radiation Injury in Prostate Cancer Patients Treated with Radiotherapy", by the Department of the Army through the Prostate Cancer Research Program synergy award mechanism. I have opened new protocols for the retrospective study of lung brachytherapy and para-spinal brachytherapy. In addition, I will be submitting in early fall an RO-1 application which proposes to prospectively study in a phase II clinical design the predictive value of testing for mutations in the ATM gene prior to the initiation of therapy.

In addition to articles, protocols and grants, I have made several presentations over the last year as an invited speaker and in order to present submitted research papers which I expect to publish in the coming year, they are enumerated in appendix B as those accepted for formal oral presentations and in appendix C as abstracts presented as either posters, poster discussion and oral presentations. In appendix D are listed research accomplishments directly related to the activities outlined in my training grant. I have included in appendix E copies of articles describing the results of my research and for which I am first author from 2007. The articles are entitled, "A genetically determined dose-volume histogram predicts for rectal bleeding among patients treated with prostate brachytherapy", "Effect of low dose-rate prostate brachytherapy on the sexual health of men with optimal sexual function before treatment: analysis at >/= 7 years of follow-up" and "Brachytherapy for Prostate Cancer" (1,2,3).

Based upon the abstracts submitted this year and the *works in progress* at this point, I expect to be able to complete the aims of my research proposal and have them largely published as outlined in the training and to report the results of further granting efforts which should sustain my research efforts into the future.

KEY RESEARCH ACCOMPLISHMENTS:

Completed 24 months of coursework required for Clinicial Research Training Program and received a Masters degree from the Mount Sinai School of Medicine.

I have published in 2007, 8 out of a total of 11, collaborative works with my clinical mentors Richard Stock, M.D., and Barry Rosenstein,PhD., regarding the natural history of prostate cancer treated with brachytherapy and the genetics of radiosensitivity.

I have established a collaborative effort with the Mount Sinai Department of Urology, which has resulted in the formulation of a research question for which we are actively seeking funding through the NIH and DOD.

I have given 7 oral presentations at major research meetings throughout the radiation and urological community in America, Japan and Germany.

I presented my findings regarding my research efforts at an oral presentation at the American Society of Therapeutic Radiation Oncology (ASTRO) annual meeting in Los Angeles, California in November of 2007, and at least partly as a result of my efforts 9 other residents, fellows and faculty presented work which I either inspired or collaborated extensively in regarding its content and formulation.

Our research team has been awarded to pursue a project entitled," Genome-Wide Association Study to Identify SNPs and CNPs Associated with Development of Radiation Injury in Prostate Cancer Patients Treated with Radiotherapy" through the Department of the Army Prostate Cancer Research Program Synergism Award Mechanism.

Presentation of my research findings in Poster format at the Innovative Minds in Prostate Cancer Today (IMPaCT) meeting September 5-7, 2007

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

10 Manuscripts in 2007 (appendix A)

7 Oral Presentations in 2007 (appendix B)

11 Abstracts in 2007 (appendix C)

CONCLUSIONS:

My professional training as a translational scientist with and emphasis on prostate cancer treatment is progressing on several important fronts. I have published several articles in medical research journals this year about prostate cancer treatment outcomes, the side effect profile of prostate cancer treatment and the genetics of radiation sensitivity.

I have completed the K30 Physician Research Training Program and have been conferred a Masters degree in May 2006 in Clinical Research from the Mount Sinai School of Medicine.

The results of my research project were presented at the ASTRO annual meeting in addition to the work of my residents, my research mentors and my junior faculty colleagues.

The research group has been awarded additional funds to study genetic associations between prostate radiotherapy and genetic polymorphisms/ mutations.

References:

1. Cesaretti JA, Stock RG, Atencio DP, Peters SA, Peters CA, Burri RJ, Stone NN, Rosenstein BS. “A genetically determined dose-volume histogram predicts for rectal bleeding among patients treated with prostate brachytherapy.” Int J Radiat Oncol Biol Phys. 2007 May 8; [Epub ahead of print]

2. Cesaretti JA, Kao J, Stone NN, Stock RG. “Effect of low dose-rate prostate brachytherapy on the sexual health of men with optimal sexual function before treatment: analysis at >/= 7 years of follow-up.” BJU Int. 2007 Aug;100(2):362-7.

3. Cesaretti JA, Stone NN, Skouteris BM, Park JL, Stock RG. “Brachytherapy for Prostate Cancer.” Cancer J. 2007 Sep-Oct;13(5):302-12.

Appendix A. - Publications in 2007:

Zagar TM, Stock RG, Cesaretti JA, Stone NN. "Assessment of postbrachytherapy sexual function: a comparison of the IIEF-5 and the MSEFS." *Brachytherapy*. 2007 Jan-Mar;6(1):26-33.

Ho AY, Burri RJ, Jennings GT, Stone NN, Cesaretti JA, Stock RG. "Is seminal vesicle implantation with permanent sources possible? A dose-volume histogram analysis in patients undergoing combined 103Pd implantation and external beam radiation for T3c prostate cancer." *Brachytherapy*. 2007 Jan-Mar;6(1):38-43.

Cesaretti JA, Stock RG, Atencio DP, Peters SA, Peters CA, Burri RJ, Stone NN, Rosenstein BS. "A genetically determined dose-volume histogram predicts for rectal bleeding among patients treated with prostate brachytherapy." *Int J Radiat Oncol Biol Phys*. 2007 May 8; [Epub ahead of print]

Ho AY, Fan G, Atencio DP, Green S, Formenti SC, Haffty BG, Iyengar P, Bernstein JL, Stock RG, Cesaretti JA, Rosenstein BS. "Possession of ATM Sequence Variants as Predictor for Late Normal Tissue Responses in Breast Cancer Patients Treated with Radiotherapy." *Int J Radiat Oncol Biol Phys.* 2007 May 19; [Epub ahead of print]

Cesaretti JA, Kao J, Stone NN, Stock RG. "Effect of low dose-rate prostate brachytherapy on the sexual health of men with optimal sexual function before treatment: analysis at >/= 7 years of follow-up." BJU Int. 2007 Aug;100(2):362-7.

Peters CA, Stock RG, Cesaretti JA, Atencio DP, Peters S, Burri RJ, Stone NN, Ostrer H, Rosenstein BS. "TGFB1 Single Nucleotide Polymorphisms are Associated with Adverse Quality of Life in Prostate Cancer Patients Treated with Radiotherapy." Int J Radiat Oncol Biol Phys. 2007 Aug 7; [Epub ahead of print]

Cesaretti JA, Stone NN, Skouteris BM, Park JL, Stock RG. "Brachytherapy for Prostate Cancer." Cancer J. 2007 Sep-Oct;13(5):302-12.

Kao J, Stone NN, Lavaf A, Dumane V, Cesaretti JA, Stock RG. "(125)I monotherapy using D90 implant doses of 180 Gy or greater." Int J Radiat Oncol Biol Phys. 2007 Oct

Lavaf A, Genden EM, Cesaretti JA, Packer S, Kao J. "Adjuvant radiotherapy improves overall survival for patients with lymph node-positive head and neck squamous cell carcinoma" Cancer. 2007 Dec 12; [Epub ahead of print]

Cesaretti J, Pennather A, Rosenstein B, Swanson S, Fernando H. "Radiosurgery for Thoracic Malignancies." Ann Thorac Surg. 2008 Jan; [In press]

Appendix B – Presentations in 2007

Cesaretti JA. "Loose versus Stranded seeds." "Genetic Predictors of Radiotherapy Response." "Salvage Brachytherapy." 12th Annual Scottsdale Prostate Cancer Symposium, March 1-4, 2007, Scottsdale, Arizona.

Cesaretti JA. "Prostate Brachytherapy, the Mount Sinai Experience." 95th Annual Japanese Urological Association meeting, April 14th 2007, Osaka, Japan.

Cesaretti JA. "Intensity Modulated Radiation Therapy for Prostate Cancer" and "Combined Modality Therapy for Prostate Cancer." Advanced Workshop in the Treatment of Prostate Cancer II, April 25-27, 2007, The New York Academy of Medicine, New York, New York.

Cesaretti JA. "Stereotactic Radiosurgery for Lung Cancer." 1st Annual Minimally Invasive Thoracic Surgery Summit, June 6th 2007, New York, New York.

Cesaretti JA. "Single Nucleotide Polymorphisms as Predictors for Development of Erectile Dysfunction in African-American Men Treated With Radiotherapy for Prostate Cancer." ASTRO 49th Annual meeting, November 2007, Los Angeles, California.

Cesaretti JA. "Brachytherapy for Prostate Cancer." New York Roentgen Society, November 2007, New York, New York.

Cesaretti JA. "Innovations in Prostate Brachytherapy." 4th International Interstitial Prostate Brachytherapy Teaching Course, January 2008, Bergisch-Gladbach, Germany.

Appendix C – Abstracts accepted for Presentations.
:

Cesaretti JA, Stock RG, Stone NN and Rosenstein BS "Combined Low Dose Rate Brachytherapy and External Beam Radiotherapy Result in a Favorable Acute Urinary Symptom Profile Relative to Brachytherapy Monotherapy at the Same Biological Equivalent Dose (BED)" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Page S630

Peters CA, Stone NN, Cesaretti JA and Stock RG "The Effect of Family History on Outcome in Patients Treated With Low-Dose Rate Brachytherapy for Clinically Localized Prostate Cancer" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S370-S371

Solan AN, Stock RG, Cesaretti JA and Stone NN "Correlation Between Erectile Dysfunction and Dose to Penile Bulb and Neurovascular Bundles Following Prostate Brachytherapy" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S351-S352

Skouteris VM, Stone NN, Stock RG and Cesaretti JA "Dose Response Study of Pd-103 Prostate Seed Implantation" International Journal of Radiation Oncology*Biology* Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S349-S350

Stock RG, Cesaretti JA and Stone NN "Comparisons of PSA Failure Definitions Following Trimodality Therapy for Intermediate to High-Risk Prostate Cancer" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S344-S345

Klein TJ, Stock RG, Cesaretti JA and Stone NN "Prognostic Significance of the 5-Year PSA Value for Predicting Prostate Cancer Recurrence Following Brachytherapy" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S176-S177

Ho AY, Fan G, Cesaretti JA, Stone NN and Stock RG "Young Men have Equivalent Biochemical Outcomes Compared to Older Men After Treatment With Prostate Brachytherapy" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S90-S91

Fan G, Skouteris B, Stone NN, Stock RG and Cesaretti JA "Impact of Prostate Volume as a Predictor of Urinary Incontinence Following Radioactive Seed Implantation for Prostate Cancer" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Page S31

Rosenstein BS, Cesaretti JA, Stock RG, Stone NN, Atencio DP, Peters CA, Burri R and Peters S "A Validation Study to Examine the Correlation Between Possession of Variants in the ATM Gene With the Development of Erectile Dysfunction in Prostate Cancer Patients Treated With Radiotherapy" International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Page S29

Moore J., Stock RG, Cesaretti JA, Stone NN, Li W, Peters S, Atencio DP, Peters CA, Burri R and Rosenstein BS "Single Nucleotide Polymorphisms as Predictors for Development of Erectile Dysfunction in African-American Men Treated With Radiotherapy for Prostate Cancer International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Page S7

Cesaretti JA, Stock RG and Rosenstein BS. "A Genetically Determined Dose Volume Histogram Predicts for Rectal Bleeding Among Patients Treated with Prostate Brachytherapy" Proceedings of the Prostate Cancer Research Program (PCRP) of Innovative Minds in Prostate Cancer Today (IMPaCT) meeting, September 2007.

Appendix D – Research efforts directly related to the training grant.

Zagar TM, Stock RG, Cesaretti JA, Stone NN. "Assessment of postbrachytherapy sexual function: a comparison of the IIEF-5 and the MSEFS." *Brachytherapy*. 2007 Jan-Mar;6(1):26-33.

Cesaretti JA, Stock RG, Atencio DP, Peters SA, Peters CA, Burri RJ, Stone NN, Rosenstein BS. "A genetically determined dose-volume histogram predicts for rectal bleeding among patients treated with prostate brachytherapy." *Int J Radiat Oncol Biol Phys*. 2007 May 8; [Epub ahead of print]

Ho AY, Fan G, Atencio DP, Green S, Formenti SC, Haffty BG, Iyengar P, Bernstein JL, Stock RG, Cesaretti JA, Rosenstein BS. "Possession of ATM Sequence Variants as Predictor for Late Normal Tissue Responses in Breast Cancer Patients Treated with Radiotherapy." *Int J Radiat Oncol Biol Phys.* 2007 May 19; [Epub ahead of print]

Cesaretti JA, Kao J, Stone NN, Stock RG. "Effect of low dose-rate prostate brachytherapy on the sexual health of men with optimal sexual function before treatment: analysis at >/= 7 years of follow-up." BJU Int. 2007 Aug;100(2):362-7.

Peters CA, Stock RG, Cesaretti JA, Atencio DP, Peters S, Burri RJ, Stone NN, Ostrer H, Rosenstein BS. "TGFB1 Single Nucleotide Polymorphisms are Associated with Adverse Quality of Life in Prostate Cancer Patients Treated with Radiotherapy." Int J Radiat Oncol Biol Phys. 2007 Aug 7; [Epub ahead of print]

Cesaretti JA, Stone NN, Skouteris BM, Park JL, Stock RG. "Brachytherapy for Prostate Cancer." Cancer J. 2007 Sep-Oct;13(5):302-12.

Kao J, Stone NN, Lavaf A, Dumane V, Cesaretti JA, Stock RG. "(125)I monotherapy using D90 implant doses of 180 Gy or greater." Int J Radiat Oncol Biol Phys. 2007 Oct 31; [Epub ahead of print]

PRESENTATIONS:

Cesaretti JA. "Loose versus Stranded seeds." "Genetic Predictors of Radiotherapy Response." "Salvage Brachytherapy." 12th Annual Scottsdale Prostate Cancer Symposium, March 1-4, 2007, Scottsdale, Arizona.

Cesaretti JA. "Prostate Brachytherapy, the Mount Sinai Experience." 95th Annual Japanese Urological Association meeting, April 14th 2007, Osaka, Japan.

Cesaretti JA. "Single Nucleotide Polymorphisms as Predictors for Development of Erectile Dysfunction in African-American Men Treated With Radiotherapy for Prostate Cancer." ASTRO 49th Annual meeting, November 2007, Los Angeles, California.

Cesaretti JA. “Brachytherapy for Prostate Cancer.” New York Roentgen Society, November 2007, New York, New York.

ABSTRACTS:

Cesaretti JA, Stock RG, Stone NN and Rosenstein BS “Combined Low Dose Rate Brachytherapy and External Beam Radiotherapy Result in a Favorable Acute Urinary Symptom Profile Relative to Brachytherapy Monotherapy at the Same Biological Equivalent Dose (BED)” International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Page S630

Peters CA, Stone NN, Cesaretti JA and Stock RG “The Effect of Family History on Outcome in Patients Treated With Low-Dose Rate Brachytherapy for Clinically Localized Prostate Cancer” International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S370-S371

Solan AN, Stock RG, Cesaretti JA and Stone NN “Correlation Between Erectile Dysfunction and Dose to Penile Bulb and Neurovascular Bundles Following Prostate Brachytherapy” International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Pages S351-S352

Rosenstein BS, Cesaretti JA, Stock RG, Stone NN, Atencio DP, Peters CA, Burri R and Peters S “A Validation Study to Examine the Correlation Between Possession of Variants in the ATM Gene With the Development of Erectile Dysfunction in Prostate Cancer Patients Treated With Radiotherapy” International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Page S29

Moore J., Stock RG, Cesaretti JA, Stone NN, Li W, Peters S, Atencio DP, Peters CA, Burri R and Rosenstein BS “Single Nucleotide Polymorphisms as Predictors for Development of Erectile Dysfunction in African-American Men Treated With Radiotherapy for Prostate Cancer.” International Journal of Radiation Oncology*Biology*Physics, Volume 69, Issue 3, Supplement 1, 1 November 2007, Page S7

Cesaretti JA, Stock RG and Rosenstein BS. “A Genetically Determined Dose Volume Histogram Predicts for Rectal Bleeding Among Patients Treated with Prostate Brachytherapy” Proceedings of the Prostate Cancer Research Program (PCRP) of Innovative Minds in Prostate Cancer Today (IMPaCT) meeting, September 2007.

Int. J. Radiation Oncology Biol. Phys., Vol. 68, No. 5, pp. 1410–1416, 2007

0360-3016/07/$–see front matter

doi:10.1016/j.ijrobp.2007.02.052

CLINICAL INVESTIGATION **Prostate**

A GENETICALLY DETERMINED DOSE–VOLUME HISTOGRAM PREDICTS FOR RECTAL BLEEDING AMONG PATIENTS TREATED WITH PROSTATE BRACHYTHERAPY

Jamie A. Cesaretti, M.D., M.S.,* Richard G. Stock, M.D.,* David P. Atencio, Ph.D.,* Sheila A. Peters, B.A.,* Christopher A. Peters, M.D.,* Ryan J. Burri, M.D.,* Nelson N. Stone, M.D.,* and Barry S. Rosenstein, Ph.D.*†‡§

Departments of *Radiation Oncology, †Community and Preventive Medicine, and ‡Dermatology, Mount Sinai School of Medicine; and §Department of Radiation Oncology, New York University School of Medicine, New York, NY

Purpose: To examine whether possession of genetic alterations in the *ATM* (ataxia telangiectasia) gene is associated with rectal bleeding in a dose-dependent and volume-dependent manner.

Methods and Materials: One hundred eight prostate cancer patients who underwent brachytherapy using either an ^{125}I implant, a ^{103}Pd implant, or the combination of external beam radiotherapy with a ^{103}Pd implant and had a minimum of 1 year follow-up were screened for DNA sequence variations in the 62 coding exons of the *ATM* gene using denaturing high-performance liquid chromatography. Rectal dose was reported as the volume (in cubic centimeters) of rectum receiving the brachytherapy prescription dose. The two-sided Fisher exact test was used to compare differences in proportions.

Results: A significant correlation between the presence of any *ATM* sequence alteration and Grade 1 to 2 proctitis was obtained when the radiation dose to rectal tissue was quantified. Rectal bleeding occurred in 4 of 13 patients (31%) with a variant versus 1 of 23 (4%) without a genetic alteration for patients who had <0.7 cm^3 of rectal tissue receiving the implant prescription dose ($p = 0.05$). Of patients in whom 0.7–1.4 cm^3 of the rectum received the implant prescription, 4 of 11 (36%) with an *ATM* alteration exhibited Grade 1 to 2 proctitis, whereas 1 of 21 (5%) without a variant ($p = 0.04$) developed this radiation-induced late effect.

Conclusions: The possession of genetic variants in the *ATM* gene is associated with the development of radiation-induced proctitis after prostate cancer radiotherapy for patients who receive the full prescription dose to either a low or a moderate volume of rectal tissue.

Genetic predictors, Adverse radiotherapy effects, DVH, Prostate cancer, Brachytherapy.

INTRODUCTION

In the treatment of prostate cancer, the efficacy of the various treatment options is of diminishing importance relative to the side-effect profiles because currently available interventions render similar disease-free survival rates (1–3). Radiation-related side effects are mediated by a number of known patient- and treatment-related factors. Patient-related characteristics including age, performance status, nutritional state, severity of diabetes, peripheral vascular disease, and the functional status of the periprostatic organs before radiotherapy are known to increase the incidence and severity of radiation-related side effects (4–6). Regarding treatment-related factors, total dose and dose rate of radiation given to the pelvis, rectum, bladder, and ejaculatory apparatus are also known to affect the incidence of side effects (7–10). Recent insight into the etiology of radiation-induced side effects was obtained from Radiation Therapy Oncology Group (RTOG) trial 94-06, which reported a difference in the incidence of late RTOG Grade 2 rectal bleeding using 1.8-Gy fractions to 79 Gy and 2.0-Gy fractions to 78 Gy; the lower dose per fraction afforded a 9% incidence, compared with a 33% incidence with the higher dose per fraction (11).

With the advent of newer technologies, there is increased interest in decreasing the incidence of side effects. With intensity-modulated radiotherapy and image-guided

Reprint requests to: Jamie A. Cesaretti, M.D., M.S., Department of Radiation Oncology, Mount Sinai School of Medicine, One Gustave Levy Place, Box 1236, New York, NY 10029. Tel: (212) 241-7818; Fax: (212) 410-7194; E-mail: jamie.cesaretti@msnyuhealth.org

Supported by Department of the Army Grant W81XWH-04-0172 (J.A.C.); American Cancer Society Research Scholar Grant RSGT-05-200-01-CCE (B.S.R.); New York State Empire Clinical Research Investigator Program Grant; New York State Department of Health Contract C017931 (B.S.R.).

Conflict of interest: J. A. C. and R. G. S. serve as consultants for CR BARD. N. N. S. has a financial interest in Prologics Inc.

Received Jan 3, 2007, and in revised form Jan 28, 2007. Accepted for publication Feb 15, 2007.

Effect of low dose-rate prostate brachytherapy on the sexual health of men with optimal sexual function before treatment: analysis at ≥7 years of follow-up

Jamie A. Cesaretti, Johnny Kao, Nelson N. Stone* and Richard G. Stock
*Departments of Radiation Oncology and *Urology, Mount Sinai School of Medicine, New York, NY, USA*

Accepted for publication 16 February 2007
This work was presented at 47th annual ASTRO annual meeting in October 2005.

OBJECTIVE

To evaluate the effect of low-dose rate prostate brachytherapy on the sexual health of men with ≥7 years of prospective evaluation and optimum sexual function before treatment.

PATIENTS AND METHODS

In all, 223 patients with T1b to T3a prostate cancer and a median (range) age of 66 (50–82) years were treated with permanent seed implantation from November 1990 to March 1998. They were followed for a median (range) of 8.2 (7–14.1) years using prospective quality-of-life measures. Erectile function (EF) was assessed using a physician-assigned score and beginning in June 2000; the validated International Index of EF (IIEF-5) was used as a complementary method to quantify late EF. No adjustment was made to differentiate sexual function with or with no pharmacological intervention for EF. Pearson's chi-square test and Student's *t*-test were used to compare the groups.

RESULTS

Of the 223 men, 131 (59%) had optimal EF before their brachytherapy; of these, 51 (40%) at the last follow-up evaluation were using either a phosphodiesterase type 5 inhibitor (44, 86%), yohimbine (two, 4%) or alprostadil (five, 10%). The age at implantation was highly predictive of current EF; 23 of 25 (92%) men aged 50–59 years had a current EF of ≥2; those aged 60–69 and 70–78 years had an EF of ≥2 in 48/75 (64%) and 18/31 (58%) ($P = 0.01$). A current IIEF-5 score of ≥16 also correlated highly with age at implant, i.e. 50–59, 16/25 (64%); 60–69, 20/75 (27%) and 70–78 years, 6/31 (19%) ($P < 0.001$).

CONCLUSION

Patients aged <60 years and with optimal EF before low-dose rate prostate brachytherapy have a very high probability of long-term EF.

KEYWORDS

prostate brachytherapy, prostate cancer, erectile dysfunction, IIEF-5

INTRODUCTION

Modern techniques for treating localized prostate cancer, including radical prostatectomy (RP), external beam radiotherapy (EBRT) and brachytherapy, have similar cancer-specific survival rates [1–3]. Treatment decisions for these patients are often difficult because of a lack of long-term toxicity data. All three treatments might result in the development of erectile dysfunction (ED), which occurs in up to 75% of patients [4–6]. Permanent ED is especially troublesome for younger and more sexually motivated men. While there are no randomized trials addressing this issue, a recent meta-analysis of non-randomized data summarized the effects of prostate cancer treatment on erectile function (EF) in 54 published articles [7]. The rate of ED after standard RP, a nerve-sparing RP, EBRT, EBRT plus brachytherapy and brachytherapy alone were 75%, 68%, 45%, 40% and 24%, respectively [7]. In addition, it is well established that the rates of ED after surgery, EBRT or brachytherapy increase with time [8,9]. Therefore, this report focuses on 131 patients with optimal EF before prostate brachytherapy who were followed for ≥7 years.

It is likely that the development of ED after prostate brachytherapy is multifactorial. Possible patient- and therapy-related factors include sexual function before treatment, age, medical comorbidities, genetic predisposition, method of data collection (patient-reported vs physician-reported), length of follow-up, dose to erectile tissues, use of hormonal therapy and use of erectile aids [10,11]. While our previous studies focused on technical and genetic predictors of brachytherapy-induced ED, the primary goal of the present study was to identify the patient-reported factors associated with late sexual dysfunction.

PATIENTS AND METHODS

Between June 1990 and March 1998, 586 men had prostate brachytherapy at Mount Sinai Hospital; the EF was followed prospectively for ≥7 years in 223 (38%) of these men, but in the remaining 363 with <7 years of follow-up for ED the many attempts to acquire the information were unsuccessful. Our practice pattern is to offer all patients a long-term prospective evaluation with several quality-of-life measures, and therefore the 223 men in the present report had chosen to continue their follow-up with the radiation oncology department rather than, or along with, their urologist.

All patients had biopsy-confirmed adenocarcinoma with the pathology reviewed at the Mount Sinai Medical Center. Patients were staged according to the 1992 American Joint Cancer Commission standard [12].

Brachytherapy for the Treatment of Prostate Cancer

Jamie A. Cesaretti, MD, MS, Nelson N. Stone, MD,* Vassilios M. Skouteris, MD,* Janelle L. Park, MD,*† and Richard G. Stock, MD**

Abstract: Low-dose rate brachytherapy has become a mainstream treatment option for men diagnosed with prostate cancer because of excellent long-term treatment outcomes in low- , intermediate- , and high-risk patients. Largely due to patient lead advocacy for minimally invasive treatment options, high-quality prostate implants have become widely available in the US, Europe, and Japan. The reason that brachytherapy results are reproducible in several different practice settings is because numerous implant quality factors have been defined over the last 20 years, which can be applied objectively to judge the success of the intervention both during and after the procedure. In addition, recent long-term follow-up studies have clarified that the secondary cancer incidence of brachytherapy is not clinically meaningful. In terms of future directions, the study of radiation repair genetics may allow for the counseling physician to better estimate any given patients risk for side effects, thereby substantially reducing the therapeutic uncertainties faced by patients choosing a prostate cancer intervention.

Key Words: prostate cancer, prostate brachytherapy, minimally invasive techniques

(*Cancer J* 2007;13: 302–312)

The use of low dose rate brachytherapy using computer-assisted treatment planning confers a reproducible cure rate with a limited side effect profile.[1] The first conception of the transperineal approach using a transrectal ultrasound probe was initially reported by Holm in 1983. Over the last 25 years numerous significant advances have been made, which built on this initial insight.[2–4] In this review we will first describe the various methods that have evolved relating to a transperineal approach. After this discussion, we will review the efficacy and side effects of prostate brachytherapy.

Outcomes derived from a broad range of brachytherapy experience, including results from large institutions with 20 years of experience, as well as results from groups reporting their initial cases, will be presented. This diversity of excellent results is arguably the main strength of prostate brachytherapy relative to the other available methods for the treatment of localized prostate cancer. Low dose rate brachytherapy pioneers carefully delineated implant quality factors, which has provided a solid foundation upon which reproducible results are possible across a spectrum of clinical experience.[5–7]

From *Department of Radiation Oncology, The Mount Sinai School of Medicine, and †Department of Radiation Oncology, James J. Peters VA Hospital, New York, NY.

Reprints: Jamie A. Cesaretti, MD, MS, 1184 5th Avenue, Box 1236, New York, NY 10029. E-mail: jamie.cesaretti@mountsinai.org.

ISSN: 1528-9117/07/1305-0302

IMPLANT TECHNIQUES

Real Time

In 1990 physicians at the Mount Sinai Medical Center developed the prostate brachytherapy technique termed the real-time method. This technique is heavily reliant on detailed clinical knowledge of the transverse and sagittal ultrasound anatomy of the prostate gland. According to the original inception of this method, an activity per volume table (nomogram) is used to find the proper amount of activity for the seeds to be implanted. Based on the concepts put forth by Patterson and Parker, a peripherally weighted implant can be completed by following a relatively straightforward set of guidelines.[4]

The first step, usually performed in the urologist's office, is determination of prostate volume by applying an ellipsoid formula (height × width × length × 0.52). This volume is used to determine the number of seeds and total activity ordered for the patient by referring to a look-up table. In the operating room, the prostate volume is remeasured using step-section planimetry at 5-mm intervals from base to apex. Three longitudinal measurements (anterior, middle, and posterior) of the prostate are made in the midline to find the average length of the gland; this important step serves as a general guide for the number of seeds to be placed within the periphery and interior of the gland. The suggested seed activities for both I-125 (range 0.3–0.6 mCi) and Pd-103 (1.5–3 U) are titrated as such to give a continuous isodose line with each other if placed no further than 1cm apart. Therefore, a prostate length of 3 cm will require 4 seeds, 1 at both the apex and base and 2 in the middle. A 4-cm length will require 5 seeds and so forth. The number of peripheral needles is determined by taking a circumferential measurement at the prostate's greatest transverse diameter. If the circumference is 12 cm, then at least 12 needles should be used. The final decision on the number of needles and spacing between needles and seeds will be somewhat dependent on the activity per seed selected. A higher activity will allow greater spacing (and therefore fewer needles and seeds) but at a cost of needing to be more conservative with proximity to the urethra and rectum. These simple measurements, in addition to referencing the look-up table, allow one to have a reliable road map for the seed implant without the use of a computer-mediated plan. In addition, it allows the implant

Appendix F

An additional goal of this study was to perform functional assays to determine the effect of *ATM* sequence variants on the function of the ATM protein. This research was accomplished using lymphoblastoid cell lines derived from EBV transformed lymphocytes obtained from five subjects who did not exhibit late responses and did not possess *ATM* genetic alterations. For experiments in which p53 phosphorylation was measured, cells were irradiated with either 0 or 4 Gy of x-rays and incubated either 0.5 or 2 hr. The densitometric results for each time point were divided by the value in each experiment for unirradiated cells to normalize these results. Each irradiation was performed a total of three times. The mean values (with standard deviations) for wild type cells incubated either 0.5 or 2.0 hr were 3.2+1.7 or 6.9+3.1, respectively. The results for the cell lines possessing variants are shown in Tables 1 and 2. In addition, ATM protein levels were measured in each cell line in three separate experiments and divided by the average value obtained for the five wild type *ATM* cell lines.

Table 1. Functional Assays of Lymphoblastoid Cells Derived from Subjects Possessing *ATM* Variants

Cell Line	Radio-sensi-tive Yes/No	Nucleotide Change	Amino Acid Substitu-tion	ATM level	Phospho-p53 0.5 hr	Phospho-p53 2 hr	Normaliz ed α-value
MS01-33	no	4138 C>T	1380 H>Y	1.1+0.6	5.1+4.4	5.4+3.0	1.2±0.2
MS01-30	No	IVS5-7 C>T 378 T>A 4578 C>T	N/A 126 D>E 1526 P>P	0.5+0.3	2.0+1.7	4.5+4.0	1.0±0.4
MS01-39	Yes	5557 G>A 5558 A>T	1853 D>N 1853 D>V	1.3±0.9	1.4±1.0	2.7±0.4	1.1±0.5
MS01-45	No	5557 G>A	1853 D>N	0.4±.04	1.4±.0.6	1.6±1.0	1.3±0.1
MS01-51	Yes	IVS5-7C>T	N/A	0.7±0.5	2.5±2.6	9.5±4.5	0.5±.2

		378 T>A	126 D>E				
MS01-37	Yes	378 T>A 1176 C>G 4138 C>T	126 D>E 392 G>G 1380 H>Y	1.6±0.2	2.1±1.2	2.0±1.2	1.4±0.4
MS01-67	Yes	4578 C>T	1526 P>P	0.5±.09	4.6±0.8	10.7±3.7	1.2±0.1
MS01-65	No	5557 G>A	1853 D>N	1.1±0.5	2.7±1.2	10.1+4.0	1.2±0.3
Ms01-53	No	378 T>A 1176 C>G	126 D>E 392 G>G	1.0±.0.1	2.5±0.8	6.5±2.1	0.8±0.3
MS01-07	No	4917 G>A 5557 G>A 5558 A>T	1639 P>P 1853 D>N 1853 D>V	0.8±0.5	0.9±0.7	2.0±1.7	0.5±0.3
MS01-37	Yes	378 T>A 1176 C>G 4138 C>T	126 D>E 392 G>G 1380 H>Y	1.6+0.2	2.1+1.2	2.0+1.2	1.4±0.2
MS02-13	YES	378 T>A 6176 C>T	126 T>A 2059 T>I	1.0+0.2	2.0+1.3	5.1+3.7	1.2±0.2
MS02-73	YES	IVS62+8 A>C	N/A	0.8+0.3	4.5+4.0	3.8+1.3	0.8±0.3
MS01-87	YES	5071 A>C	1691 S>R	1.0+0.5	2.3+1.3	5.0+2.0	0.8±0.1
MS01-03	NO	2614 C>T	872 P>S	0.7+0.2	1.1+0.8	1.5+0.4	1.2±0.6

		2685 A>C	895 L>L				
MS01-35	NO	1229 T>C	410 V>A	0.9+0.6	2.9+0.1	5.7+3.7	1.1±0.2
MS02-34	YES	915 G>C	25 R>P	2.0+1.5	1.5+0.6	1.8+1.1	1.3±0.5
MS02-05	YES	NONE	N/A	0.7+0.4	3.1+3.7	4.6+3.7	1.1±0.3
MS03-13	YES	NONE	N/A	0.7+0.1	3.6+1.2	7.9+3.8	0.9±0.5
MS03-48	YES	NONE	N/A	0.5+0.3	2.4+1.4	6.8+2.1	1.3±0.1

Table 2. Functional Assays of *ATM* Homozygoyte and *ATM* Heterozygote Lymphoblastoid Cell Lines

Cell Line	Homozygote or Heterozygote	ATM Level	Phospho p53 0.5 hr	Phospho p53 2 hr	Normalized α-value for radiation survival curve
8388	heterozygote	0.7±0.6	1.6±0.2	6.7±2.3	1.5±0.2
8925	heterozygote	0.7±0.8	1.9±0.4	5.1±0.1	1.4±0.2
8928	heterozygote	0.8±0.3	3.8±3.5	3.5±2.7	1.7±0.2
9579	heterozygote	0.5±0.3	2.3±1.3	2.6±0.3	1.1±0.3
2781	heterozygote	0.7±0.5	3.2±0.6	4.5±4.1	1.6±0.2
9588	heterozygote	0.5±0.5	6.1±4.0	6.9±2.8	1.2±0.3
8436	homozygote	0.04±0.06	2.9±1.2	2.8±0.4	1.8±0.3
9581	homozygote	0.08±0.02	1.5±1.7	4.0±1.6	2.0±0.3
9582	homozygote	0.05±0.02	2.0±4.4	2.1±0.4	2.2±0.3
2782	homozygote	0.08±0.05	2.1±3.1	3.1±1.3	2.1±0.3
1525	homozygote	0.05±0.02	2.6±1.1	3.1±1.2	1.8±0.2
11254	homozygote	0.09±0.06	1.8±0.1	2.5±0.9	2.3±0.3
9586	homozygote	0.24±0.22	1.7±1.0	4.3±1.9	1.8±0.4
13328	homozygote	0.13±0.09	0.6±0.5	2.1±1.3	2.1±0.3

The results for cells derived from AT patients clearly show a significantly lower level of ATM protein in these cells compared with wild type cells. In addition, the levels of p53 phosphorylation are consistently lower than those detected in wild type cells. The ATM levels are also consistently lower in the heterozygotes and the levels of phosphorylated p53 are also generally lower, although none of these values differed significantly from those obtained for wild type cells due to the variation in the results between experiments. There was a variation among the cell lines, but no clear pattern emerged that correlated either with the possession of an *ATM* variant (including the 5557 SNP) or whether the patient developed a late radiotherapy reaction. Hence, the results of this work suggest that

neither measurement of ATM levels nor p53 phosphorylation can serve as a predictor as to whether the patient will develop late morbidity following radiotherapy.

The radiosensitivity of each cell line was also determined from the growth response of cells irradiated with either 0, 0.5, 1.0 or 2.0 Gy of X-rays by extrapolating the growth curve to the intercept at zero time. The radiosensitivity of each cell line was estimated from the α-value ($S = e^{-\alpha D}$) normalized to the value obtained for wild type cells listed in Tables 1 and 2. The α-values for the cell lines derived from AT patients were all significantly greater than one. In addition, the α-values for the AT heterozygotes were consistently greater than one, although generally not significantly greater. In contrast, the α-values for the cell lines obtained from the breast cancer patients were variable and none was significantly greater than one.

This is not altogether surprising, since clearly none of the patients screened in this study manifested a radiation sensitivity approaching that displayed by a person suffering from AT. Any radiosensitive patients likely have only a mild radiosensitivity. However, even a slight radiosensitivity is probably sufficient to result the development of a late response since the dose used in treating breast cancer represents the tolerance dose. Hence, even just a 5-10% increase in radiosensitivity will make the difference as to whether a person will or will not develop a radiation complication. It is likely that the subtle changes in ATM protein function that result from the variants identified in this study are sufficient to cause these types of very mild changes in protein function. In contrast, it is impossible with the techniques currently available to detect such small changes in ATM function using the westerns performed in this work to measure ATM levels and p53 phosphorylation. Hence, the results of this study indicate that the identification of genetic variants will serve as a far more important basis of a predictive assay for radiosensitivity compared with functional assays.

Key Research Accomplishments

- No significant differences were detected in any of the functional end-points measured between patients who developed late complications compared with those that did not exhibit this type of radiation-induced morbidity. In addition, no significant differences in the results for the functional assays were identified for any *ATM* variant compared with wild type cells.